

W56:

5' - AAACAGGGACCCATATGGAAGACGC - 3' (SEQ ID NO: 34)

W57:

5' - AATTAACGAGGAATTCGTCATCGCTGAATACAG - 3' (SEQ ID NO: 35)

Please delete the paragraph on page 19, lines 1-11, and replace it with the following paragraph:

Example 3

Preparation of further triple mutant enzyme

The following primers were used to create the triple mutant T214A/I232A/E354K using a standard PCR reaction and with the pET23 plasmid with the T214A mutation as template:

CTGATTACACCCAAGGGGATG (SEQ ID NO: 26)	E354K-sense
CATCCCCCTTGGGTGTAATCAG (SEQ ID NO: 27)	E354K-antisense
GCAATCAAATCGCTCCGATACTGC (SEQ ID NO: 30)	I232A-sense
GCAGTATCCGAGCGATTTGATTGC (SEQ ID NO: 31)	I232A-antisense.

Please delete the paragraph on page 19, lines 13-31, and replace it with the following paragraph:

Example 4

Identification of thermostable 295 mutant

The F295 mutant was created using the error-prone PCR method described by Fromant et al., Analytical Biochemistry, vol 224, 347-353 (1995). The PCR conditions used were as follows:

0.5 µl (50 ng) plasmid pET23  
5.0 µl 10x KCI reaction buffer  
1 µl primer 1 - 60 pmoles of each primer  
1 µl primer 2  
1 µl Biotaq™ polymerase (5U)  
2 µl dNTPs, in mixture 35 mM dTTP, 12.5 mM dGTP, 22.5 mM dCTP,  
14 mM dATP

1.76  $\mu$ l  $MgCl_2$  (50 mM stock)

1  $\mu$ l  $MnCl_2$  (25 mM stock) [final concentration in reaction = 3.26  
nM]

36.7  $\mu$ l  $dH_2O$

Primer 1 = 5' - AAACAGGGACCCATATGGAAGACGC - 3' (SEQ ID NO: 34)

Primer 2 = 5' - AATTAAC TCGAGGAATTCGTCATCGCTGAATACAG -3' (SEQ ID NO:  
35)

Please delete the paragraph on page 21, lines 1-10, and replace it with the  
following paragraph:

Example 5

Other mutants of the invention were produced by PCR using  
appropriate combinations of the oligonucleotides listed above as  
well as the following:

GAAAGGCCCGGCACCAGCCTATCCTCTAGAGG (SEQ ID NO: 5) F14A-sense

CCTCTAGCGGATAGGCTGGTGCCGGGCCTTTC (SEQ ID NO: 6) F14A-antisense

GAGATACGCCGCGGTTCTG (SEQ ID NO: 9) L35A-sense

CCAGGAACCGCGGCGTATCTC (SEQ ID NO: 10) L35A-antisense

Insert the attached Sequence Listing in place of the Sequence Listing submitted  
April 29, 2002.